

## Water-Proofing Properties of Cuticular Lipids<sup>1</sup>

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**SYNOPSIS.** Epicuticular lipids play a critical role in allowing arthropods to thrive in terrestrial environments, by reducing transpiration of water through the cuticle. These lipids consist of a diverse array of compounds, especially long-chain hydrocarbons. Rates of water loss are correlated with hydrocarbon structural features, including chain length, unsaturation and methyl-branching. The water-proofing abilities of cuticular lipids appear to depend largely on their physical properties. In most arthropods, rates of water loss increase rapidly above a “transition” temperature. A widely accepted model proposes that this transition is due to melting of the surface lipids to a fluid, permeable state. Evidence for this hypothesis has primarily been correlative, due to experimental limitations. Recent technical advances in lipid biophysics and water loss measurements have made it possible to test the lipid melting model more directly. Experiments using model cuticles, *in vitro* preparations and intact arthropods support the idea that the phase behavior of cuticular lipids is a major factor determining cuticular permeability.

### INTRODUCTION

All terrestrial organisms face the problem of evaporative water loss. Insects and other arthropods are particularly vulnerable, due to their relatively small size. Transpiration through the cuticle is the main route of water loss from insects (Hadley, 1994a), so physiologists have long been interested in cuticular mechanisms for water conservation. The primary passive barrier to evaporative water loss is a thin layer of lipids on the surface of the cuticle. These lipids are highly diverse, sometimes including over 100 different compounds on a single individual (Blomquist *et al.*, 1987; Lockey, 1988; de Renobales *et al.*, 1991). The primary purpose of this review is to discuss the physiological consequences of surface lipid diversity: How do differences in lipid composition affect water balance in terrestrial arthropods? The most widely accepted model is that the water-proofing abilities of cuticular lipids depend upon their physical properties, which depend in turn upon their

chemical composition. Thus, studies of the physical properties of cuticular lipids will be emphasized.

### THE TRANSITION TEMPERATURE PHENOMENON

Rates of water loss from terrestrial arthropods are very temperature-dependent. A common observation has been that water loss from intact insects is relatively slow at moderate temperatures, then increases rapidly above a “critical” or “transition” temperature ( $T_c$ ) (Wigglesworth, 1945; Loveridge, 1968; Davis, 1974; Toolson *et al.*, 1979; Hadley and Quinlan, 1989; Fig. 1). Similar results have been obtained using excised patches of cuticle (Machin and Lampert, 1989), indicating that this transition is due to a change in the permeability characteristics of the cuticle. Because of the importance of surface lipids in reducing cuticular permeability, it was suggested as early as the 1930s (Ramsay, 1935) that the transition in water loss rate is due to a change in the properties of the lipid layer. Specifically, it was hypothesized that surface lipids melt at the  $T_c$ , resulting in their conversion from a solid, impermeable barrier to a fluid state through which water can easily diffuse. Thus, the transition temperature for water loss would result from an

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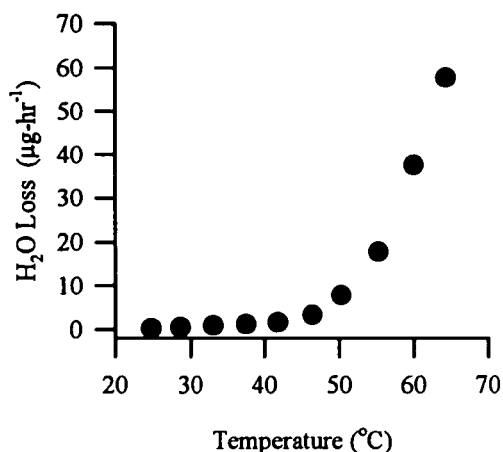


FIG. 1. Effects of temperature on water loss from a grasshopper, *Melanoplus sanguinipes*.

actual phase transition of the cuticular lipids. Early analyses of lipid properties using capillary melting point techniques provided correlative support for this model (Beament, 1945).

A historically important idea was the monolayer hypothesis proposed by Beament (1958, 1964). This model postulated a particular molecular orientation and packing for the surface lipids, based on the idea that surface lipids are amphipathic (*i.e.*, lipid molecules have both polar and non-polar portions). Under the monolayer hypothesis, polar ends of lipid molecules interact with the cuticle, while hydrophobic tails align themselves in a manner which maximizes van der Waals interactions and minimizes water flux. Above the  $T_c$ , the tails would change their orientation so that water could pass through more easily. The monolayer hypothesis never received any experimental support and is incompatible with subsequent chemical analyses of surface lipids, which revealed that nearly all cuticular lipids are entirely non-polar. However, this proposal served to focus attention on the importance of the physical properties of surface lipids, particularly their phase behavior.

#### COMPOSITION OF CUTICULAR LIPIDS

Gas chromatography-mass spectrometry analyses of cuticular lipids have become routine in many laboratories. The extensive

literature devoted to lipid composition has been well reviewed in recent years (Blomquist *et al.*, 1987; Lockey, 1988; de Renobales *et al.*, 1991; several chapters in Stanley-Samuelson and Nelson, 1993), so only a brief outline will be presented here. In most arthropod species studied to date, long-chain hydrocarbons are the predominant constituents. Hydrocarbons typically range in length from 21 to >40 carbons, and often contain one or more double bonds or methyl branches. Oxygenated lipids such as wax esters and ketones also occur and have recently begun to receive greater attention (Buckner, 1993). Phospholipids are rarely found on the surface of arthropods, and their presence may result from contamination from internal membranes.

Variation in the composition of surface lipids exists at all levels of organization. Specific classes of lipid predominate in some taxa (*e.g.*, alkenes in *Drosophila*; Bartelt *et al.*, 1986), and cuticular lipids have proven to be useful taxonomic characters (Lockey, 1991). Within a species, lipid composition varies among populations, and among colonies of social insects (Howard, 1993; Singer, 1998). Surface lipids may change from one developmental stage to another, or may change as an individual reaches reproductive maturity and produces cuticular pheromones (Nelson *et al.*, 1981). Lipid composition is also affected by temperature and other environmental factors (Hadley, 1977; Toolson and Hadley, 1979; Toolson, 1982; Gibbs and Mousseau, 1994; Howard *et al.*, 1995).

#### LIPID COMPOSITION AND WATER LOSS RATES

Several inter-specific comparisons of cuticular lipid composition have demonstrated correlations between lipid structure and water loss rates. The general pattern is that species from warmer, drier habitats lose water less rapidly than mesic species, and have longer chain-length hydrocarbons (Toolson and Hadley, 1977; Hadley, 1978). Within a given species, correlations between lipid composition and water loss follow much the same pattern (Hadley, 1977; Toolson and Hadley, 1979; Toolson, 1982; 1984). Increases in hydrocarbon unsaturation and

methylbranching have also been associated with higher water loss (Hadley, 1978; Toolson, 1984; Hadley and Schultz, 1987).

The proposed mechanistic link between lipid composition and water loss is the physical properties of the surface lipids:

Lipid composition  $\Rightarrow$  Physical properties  
 $\Rightarrow$  Water loss

Longer chain hydrocarbons will tend to melt at higher temperatures, so the studies outlined above are consistent with the idea that higher lipid melting points will result in reduced water loss.

Studies correlating lipid composition with rates of water loss have not provided a rigorous test of the lipid phase transition model, for several reasons. Lipid physical properties were not measured directly, but were inferred by analogy with other lipids, usually membrane phospholipids. The properties of phospholipids are largely dependent upon interactions between their polar headgroups and water (Crowe *et al.*, 1992), so these compounds may be inappropriate models. Second, if surface lipids from all species or treatment groups were in the same phase state, it is unclear why rates of water loss should differ. Quantities of surface lipids vary among species and individuals, so that differences in transpiration may reflect differences in the thickness of the lipid layer, rather than biophysical differences. Finally, in most studies rates of water loss were measured at only a single temperature. One would expect the  $T_c$  to differ among species or individuals with differing lipid composition, but this can only be determined by measuring water loss rates over a range of temperatures.

#### PHYSICAL PROPERTIES OF CUTICULAR LIPIDS

Relatively few biophysical studies of cuticular lipids have been performed. One reason for the scarcity of studies is the limited quantities of lipid available. Cuticular lipids typically comprise only about 0.1% of the total mass of insects, so lipids from many specimens must be combined for analyses. Information on individual variation in lipid properties is therefore lost. A second factor is the complexity of surface lipids. Some techniques (for example, differential scan-

ning calorimetry, or DSC) are well suited for studying phase transitions and other properties in homogeneous samples, but may not work well with mixtures that melt over a wide range of temperatures. One way to get around these problems is to examine pure compounds and simple mixtures, as models for naturally-occurring surface lipids.

#### *Physical properties of pure hydrocarbons and model mixtures*

Depending upon their chemical structure, lipids can undergo a variety of phase transitions, of which melting from a solid to a liquid phase is only one (Cevc, 1991). Most arthropods contain straight-chain alkanes with >20 carbons, which melt above 35°C (Gibbs and Pomonis, 1995). Below the melting point, pure *n*-alkanes can exist in several crystalline states with different packing arrangements (Small, 1986). In all such conformations, the alkyl chains are fully extended and parallel to one another. As the temperature is increased, motions of  $-\text{CH}_2-$  moieties near the ends of the chains increase (Maroncelli *et al.*, 1982). Above the melting temperature ( $T_m$ ), molecular motion increases further, and *trans-gauche* isomerizations of carbon-carbon bonds put kinks in the alkyl chains and disrupt packing.

Addition of a methyl branch interferes with hydrocarbon packing in the crystalline state. Thus, methylalkanes melt at lower temperatures than *n*-alkanes of the same chain length. The reduction in  $T_m$  depends on the location of the methyl group, with internally-branched hydrocarbons having lower  $T_m$  values (Fig. 2). Unsaturation also reduces  $T_m$ . All double bonds of cuticular hydrocarbons are in the *cis* conformation, which introduces a permanent 30 degree kink in the molecule. This disrupts packing, and alkenes melt  $\sim 50^\circ\text{C}$  below the  $T_m$  of *n*-alkanes having the same chain length (Gibbs and Pomonis, 1995).

These studies demonstrate that pure hydrocarbons can melt at biologically relevant temperatures, but arthropod cuticles contain dozens of different compounds with widely varying melting temperatures. Very little information on interactions between hydro-

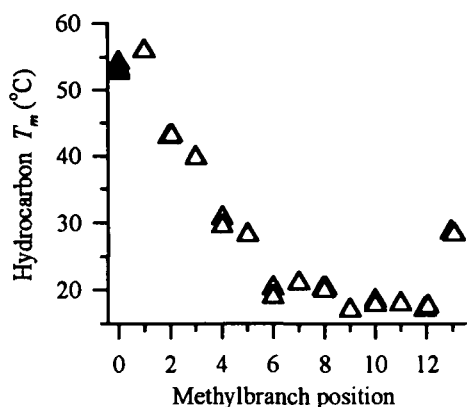


FIG. 2. Effects of methylbranch position on  $T_m$  of synthetic methylpentacosanes. Reprinted with permission from *Comparative Biochemistry and Physiology*, 112, A. Gibbs and J.G. Pomonis, Physical properties of insect cuticular hydrocarbons: the effects of chain length, methyl-branching and unsaturation, 243–249, 1995, Elsevier Science Inc.

carbons is available. Simple mixtures of two *n*-alkanes with different chain lengths, or of an *n*-alkane and a methylalkane, melt at temperatures equal to the weighted average of the component molecules (Gibbs, 1995). As long as the chain lengths are not too different, *n*-alkanes appear to pack well in the solid phase, so that the mixed crystalline lattice melts at an intermediate temperature (Bonsor and Bloor, 1977).

Mixtures of alkanes with alkenes exhibit more complex behavior. Gibbs (1995), using infrared (IR) spectroscopy, found that the  $T_m$  for these mixtures was as much as 17°C higher than the weighted average of the  $T_m$  values of the components (Fig. 3), and concluded that interactions between the compounds increased the  $T_m$ . A different interpretation was provided by Small (1986), who used DSC, X-ray diffraction and nuclear magnetic resonance to study similar mixtures. He concluded that the alkane and alkene were miscible when melted, but completely immiscible in the solid phase. Both compounds would exist as distinct crystals at low temperatures, with the alkene melting at its normal  $T_m$ . At intermediate temperatures, the alkane would begin to melt and mix with the liquid phase alkenes. The temperature at which melting is complete would depend upon the relative

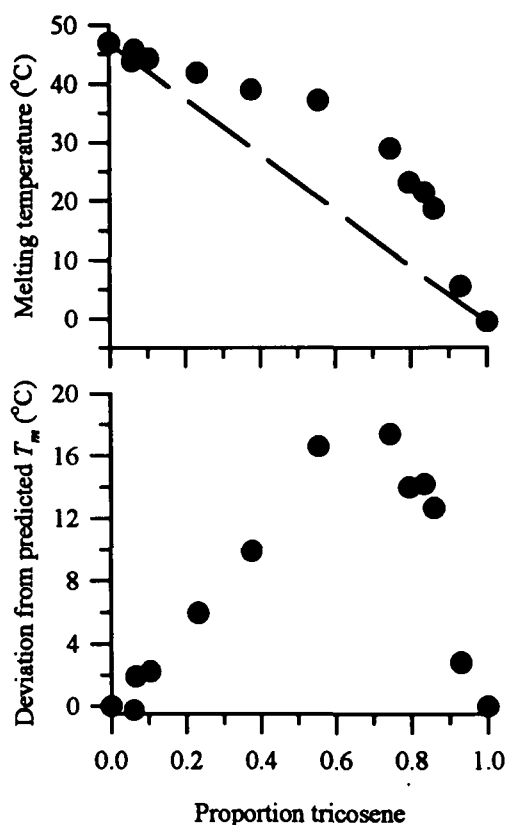


FIG. 3. A. Melting temperatures of lipid mixtures containing 23-carbon *n*-tricosane and (*Z*)-9-tricosene. Dashed line indicates  $T_m$  value predicted if the  $T_m$  of the mixture equalled the weighted average of the  $T_m$  values of the component hydrocarbons. B. Data from panel A re-plotted as the difference between the measured  $T_m$  and the predicted value. Reprinted with permission from *Comparative Biochemistry and Physiology*, 112, A. Gibbs, Physical properties of insect cuticular hydrocarbons: model mixtures and interactions, 667–672, 1995, Elsevier Science Inc.

proportions of the hydrocarbons. An interesting physiological implication is that species (such as *Drosophila*) which have significant levels of alkenes may have coexisting regions of solid- and liquid-phase lipids. In cell membranes, boundaries between gel- and fluid-phase regions are thought to be more permeable than homogeneous regions (Crowe *et al.*, 1992). If this is also true for surface lipids, then phase separation may be important in cuticular transpiration.

#### Physical properties of cuticular lipids

Naturally-occurring lipids are much more diverse than the simple mixtures discussed

so far. With the exception of a few IR spectroscopy studies, biophysical analyses have not directly addressed the relationship between lipid composition and physical properties. The first biophysical studies were performed by Beament (1945), who extracted lipids from a variety of insects and measured their melting temperatures using the capillary melting point method. The  $T_m$  values for several species of arthropod were close to their transition temperatures for water loss. Since then, lipid melting has been the major focus of physiologists interested in cuticular water loss, and other lipid properties have received little attention.

Several other biophysical techniques have been applied to surface lipids. A study using electron reflection and electron diffraction found no evidence for changes in the crystal structure of mealworm wax at the  $T_c$  (Holdgate and Seal, 1956). However, capillary melting point measurements indicated that melting occurred without observable effects on unit cell dimensions, so the interpretation of the electron diffraction patterns is uncertain. Lockey (1976) examined the effects of melting on the permeability of fatty acid monolayers, which form spontaneously on the surface of water. Fatty acids are minor components of cuticular lipid mixtures and may sometimes be contaminants from internal sources (Buckner, 1993). Thus, these results and studies of phospholipid bilayers may be irrelevant with respect to cuticular permeability. Experiments using cuticular lipids isolated from cockroaches confirmed that these compounds do not form tight films on water surfaces (Lockey, 1976).

Electron spin resonance (ESR) has been used to investigate the properties of scorpion lipids *in situ* (Toolson *et al.*, 1979). Techniques like ESR, which use lipid probes applied to the sample, are problematic if the probe does not partition evenly among different sections of the cuticle, although partitioning did not seem to present a problem in this study. Two results are of interest here. First, ESR provided information on the orientation of the probe in the surface lipids. No preferred direction was observed, suggesting that cuticular lipids in which it was placed do not adopt a pre-

ferred orientation, in contrast to the monolayer hypothesis proposed by Beament (1964). Second, the mobility of the spin-labelled probe increased sharply above 30°C, whereas the transition temperature for water loss was over 60°C. The probe was very mobile even at the lowest temperatures used, so that differences in lipid properties at higher temperatures may not have been detectable using ESR. The physical basis for the change in mobility is unclear and may reflect some process other than lipid melting.

Differential scanning calorimetry (DSC) is widely used in biophysical analyses of lipid systems. Machin and Lampert (1990) used DSC to study the thermal properties of patches of wings isolated from the American cockroach, *Periplaneta americana*. Intact wings exhibited a major endotherm, peaking at about 10°C. Lipids extracted from *P. americana* revealed two endotherms, peaking at approximately 7 and 25°C. Unfortunately, the procedures used would have extracted both surface and internal lipids, so it is impossible to attribute either of these peaks specifically to heat absorption by cuticular lipids. It is interesting to note that the  $T_c$  for this species is ~30°C (unpublished data), close to one of the endotherms. However, two factors limit the general use of DSC for cuticular lipid analyses. First, DSC typically requires relatively large (milligram) samples which may be unavailable. Second, lipid mixtures have broad phase transitions, and melting endotherms may be difficult to distinguish from background heat absorbance.

Infrared spectroscopy has several advantages for the study of gel-fluid phase transitions: (1) no probe molecules are used, (2) the signal is cumulative, and even strong background IR absorbance can be subtracted, so that (3) samples as small as a few micrograms can be analyzed. Studies using IR spectroscopy reveal that general correlations between lipid structure and physical properties found in pure compounds also exist in surface lipids of insects. For example, as female houseflies reach reproductive maturity, they produce an unsaturated (low- $T_m$ ) pheromone, and the melting point of the total surface lipid decreases (Gibbs

*et al.*, 1995). In grasshoppers (*Melanoplus sanguinipes*), acclimation to higher temperatures results in reduced levels of branched alkanes and higher  $T_m$  values (Gibbs and Mousseau, 1994). This occurs in spite of the fact that *n*-alkanes from *M. sanguinipes* tend to be shorter than methylalkanes. It appears that the higher  $T_m$  of straight-chain alkanes (Gibbs and Pomonis, 1995) more than offsets the effects of their shorter chain length. In the desert fruit fly, *D. mojavensis*, thermal acclimation results in longer-chain hydrocarbons (primarily alkenes), but no change in  $T_m$  (Gibbs *et al.*, 1998). In this case, chain elongation and unsaturation appear to offset each other. These results demonstrate that chain length alone is a rather poor indicator of  $T_m$ .

Despite the scarcity of biophysical studies, one very important conclusion can be drawn. Lipid melting and other changes in the physical properties of cuticular lipids can occur at environmentally-relevant temperatures. This is clearly the case for *P. americana*, which has long been a popular study organism because of its "greasy" cuticle. Infrared measurements reveal that surface lipids from this species melt at 27–33°C (unpublished data), and those from *M. sanguinipes* and desert *Drosophila* melt at ~40°C, temperatures frequently found in their natural habitats. Thus, phase transitions of surface lipids may have physiologically significant effects in nature.

#### LIPID PHYSICAL PROPERTIES AND WATER LOSS RATES

Attempts to relate the physical properties of surface lipids to cuticular permeability have been infrequent and sometimes contradictory. Several factors contribute to the disagreement. Cuticular permeability can vary from one region of the cuticle to another, and other routes for water loss (*e.g.*, respiration) may be important, especially at higher temperatures. Each lab generally develops and builds its own equipment for measuring rates of water loss, and they study different species of arthropod. As noted above, some biophysical methods lack the sensitivity needed for small lipid samples, or may detect physical changes unrelated to the water-proofing properties of the

surface lipids. In addition to these experimental considerations, the manner in which data are reported has been inconsistent and controversial.

Most studies have expressed rates of water loss as water lost per unit surface area per unit time, plotted against temperature on the abscissa. Because the saturated vapor pressure of water increases dramatically with temperature, water loss rates are often "corrected" by dividing by the vapor pressure deficit (*i.e.*, the difference in vapor pressure between water-saturated air and the actual water vapor content). The need for this correction was challenged by Toolson (1978, 1980), who argued that the driving force for transpiration through the cuticle is the difference in the chemical activity of water. In this case, the gradient for transpiration would be the difference between the internal environment (activity of water  $\approx 1$ ) and the external environment (activity  $\approx$  relative humidity/100), and correction for vapor pressure deficit would be inappropriate. Toolson's arguments have faced strenuous disagreement (Monteith and Campbell, 1980). Recent theoretical work promises to resolve some of these disputes, by taking into consideration some of the complexities of cuticular transpiration (Gelman *et al.*, 1988; Gelman and Machin, 1994). Water molecules must diffuse from the hemolymph into the cuticle, cross the cuticle, and then enter the gaseous phase. Also, water itself undergoes a liquid-vapor phase transition which greatly complicates the analysis.

An additional problem is the fact that critical temperatures are often estimated by visual inspection of plots of water-loss rate vs. temperature. The value of  $T_c$  can depend on the absolute magnitude of the graphical scale, no matter what units are used (Holdgate and Seal, 1956; Toolson, 1978). Some problems may be avoided with the use of Arrhenius plots ( $\ln$ [water loss] vs.  $1/T$ ). Despite their wide use in other fields and solid theoretical foundation (Berry *et al.*, 1980), Arrhenius plots have enjoyed only sporadic use in the area of insect water balance (Chefurka and Pepper, 1955; Holdgate and Seal, 1956; Toolson, 1980; Machin and Lampert, 1989). Figure 4 depicts the effects

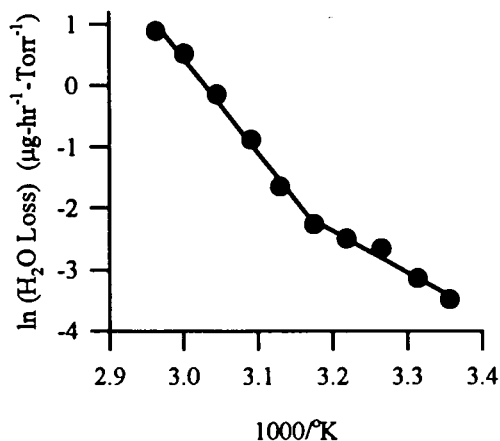


FIG. 4. Arrhenius plot of the effects of temperature on water loss from a grasshopper (*Melanoplus sanguinipes*). The grasshopper was killed with cyanide vapor, and water loss was measured continuously as the decrease in mass at each temperature.

of temperature on water loss rates from a freshly-killed grasshopper, plotted with correction for vapor pressure deficit. The plot is biphasic, with a break point at 42°C. An identical break-point is obtained when the same data are plotted without correction for vapor pressure deficit. Because this correction is simply a linear transformation on an Arrhenius plot, the slopes on either side of the break point change, but the temperature at which they intersect does not. Thus, use of Arrhenius plots effectively side-steps the issue of the driving force for water loss, since correction for vapor pressure deficit does not affect the estimate of the transition temperature. One can then focus on the question: Is the transition phenomenon due to melting of cuticular lipids? Researchers have used several approaches: model cuticle techniques, in which water flux through a lipid-coated membrane is measured, *in vitro* preparations and whole organism experiments.

#### *Model cuticle and in vitro studies of cuticular permeability*

Insects and other terrestrial arthropods lose water via several routes, including respiration, excretion and transpiration through the cuticle. Ideally, one would like to distinguish cuticular transpiration from other avenues of water loss, and then demonstrate

that melting of surface lipids causes a transition in cuticular water loss. However, both lipid properties and rates of water loss may differ from one region of the body to the next (Gibbs and Crowe, 1991; Hadley *et al.*, 1989), making it difficult to draw definitive conclusions.

Measurements of water flux through isolated pieces of cuticle can reduce complications associated with regional variation (Loveridge, 1980; Hadley and Quinlan, 1987; Machin and Lampert, 1989). In addition, these systems provide the opportunity to modify the amount and composition of the surface lipids. Thus, patches of cuticle or model cuticles made from other materials can be used to test hypotheses about cuticular lipid function in a more direct manner. A variety of these *in vitro* studies have been performed (Loveridge, 1980), although very few have examined the relationship between surface lipids and permeability. In one such effort, an Arrhenius plot of the permeability of discs of pronotum from *P. americana* exhibited a transition ~25°C (Machin and Lampert, 1989). This is close to the  $T_c$  for the intact animal and the  $T_m$  (~30°C) for cuticular lipids from this species. Thus, these experiments support a causal relationship between lipid melting and changes in cuticular permeability.

Beament (1945) measured rates of water flux through butterfly (*Pieris*) wings, which had been extracted with chloroform and coated with lipids isolated from a variety of insects. The apparatus used in these experiments was fairly crude and suffered numerous problems (Loveridge, 1980). Also, Beament later indicated that both the lipids and the cuticular substrates used were up to several years old (Beament, 1958). In spite of these difficulties, these preparations exhibited transitions in permeability at the same temperatures as intact specimens (Beament, 1945; Wigglesworth, 1945).

We have recently measured water flux through synthetic membranes coated with pure commercial hydrocarbons. Figure 5 shows an Arrhenius plot of the effects of temperature on water flux through a membrane coated with *n*-docosane, which melts at 44°C. The transition in permeability close

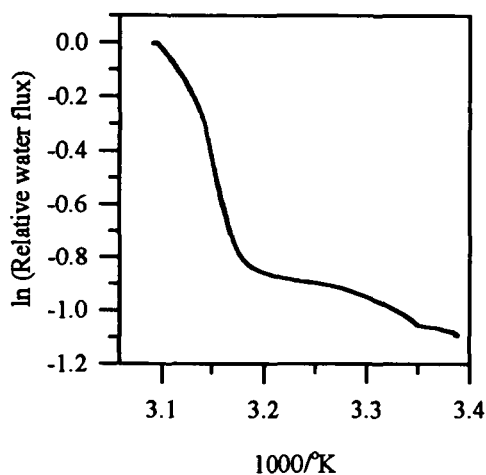


FIG. 5. Effects of temperature on water flux through a model cuticle. A Gore-Tex membrane was coated with *n*-docosane ( $T_m = 44^\circ\text{C}$ ), and water flux was measured using a flow-through system.

to the  $T_m$  of the hydrocarbon suggests a causal relationship between lipid melting and increased water flux. This could be mere coincidence, but experiments using hydrocarbons with varying  $T_m$  values produce similar results (Rourke, Firouznam, and Gibbs, in prep).

Other studies have not always supported the lipid melting model. For example, Hadley *et al.* (1982) coated a portion of scorpion cuticle with *n*-eicosane, a hydrocarbon which melts at  $38^\circ\text{C}$ . No evidence of a transition in permeability was observed over  $25\text{--}42^\circ\text{C}$ . However, this preparation was much more permeable than the cuticle of intact scorpions and may have been damaged during handling. Also, cuticular lipids were not removed prior to treatment with eicosane and may have continued to provide the main barrier to water loss.

#### *Organismal water loss and lipid properties*

The phenomenon of the critical temperature is robust; transitions in rates of water loss have been observed in numerous arthropods (Edney, 1977; Hadley, 1994a). However, physical properties of surface lipids been examined in only a few species. Table 1 lists several insects for which both  $T_c$  and lipid  $T_m$  values have been measured. Rates of water loss were measured for dead

TABLE 1. Transition temperatures ( $T_c$ ) and lipid melting temperatures ( $T_m$ ) for insects.

Species	$T_c$ ( $^\circ\text{C}$ )	$T_m$ ( $^\circ\text{C}$ )
<i>Periplaneta americana</i> (cockroach)	30 <sup>1</sup> 25 <sup>3</sup>	27–33 <sup>2</sup>
<i>Blattella germanica</i> (cockroach)	30–35 <sup>4</sup>	33 <sup>2</sup>
<i>Melanoplus sanguinipes</i> (grasshopper)	37–42 <sup>2</sup>	38–50 <sup>5</sup>
<i>Nematus ribesii</i> (sawfly larva)	35 <sup>4</sup>	36–42 <sup>6</sup>
<i>Pogonomyrmex rugosus</i> (ant worker)	42 <sup>7</sup>	38–43 <sup>2</sup>
<i>Rhodnius prolixus</i> (kissing bug)	55–60 <sup>4</sup>	60.5 <sup>6</sup>
<i>Pieris</i> spp. (butterfly larva)	40 <sup>4</sup>	67–>100 <sup>6</sup>
<i>Tenebrio molitor</i> (mealworm)	50–55 <sup>4</sup>	$\geq 80$ <sup>8</sup>

<sup>1</sup> Beament, 1958.

<sup>2</sup> Gibbs, unpublished data.

<sup>3</sup> Machin and Lampert, 1989.

<sup>4</sup> Wigglesworth, 1945.

<sup>5</sup> Gibbs *et al.*, 1991.

<sup>6</sup> Beament, 1945.

<sup>7</sup> Lighton and Feener, 1989.

<sup>8</sup> Gibbs and Crowe, 1991.

specimens, to prevent respiratory or excretory water loss. At moderate temperatures, cuticular transpiration often accounts for >90% of overall water loss (Hadley, 1994b), but the relative contribution of respiratory water loss may increase due to the direct effects of temperature and increased activity related to stress. Use of dead insects is based on the assumption that water is lost only through the cuticle, at the same rate as when alive. This may be true for some arthropods (Hadley and Quinlan, 1989; Lighton and Feener, 1989), but others may actually lose water faster after death (*e.g.*, *Drosophila*, unpublished observations). Non-cuticular routes for water loss can be blocked by sealing respiratory and digestive openings, although care is necessary to avoid damaging the insect (Hadley *et al.*, 1982; Hadley and Quinlan, 1989). Flow-through respirometry systems capable of distinguishing respiratory from cuticular water loss, at least in some arthropods, have been developed (Lighton, 1994), but have not yet received wide use in temperature studies.

Correlations between  $T_c$  and  $T_m$  have three possible results: the transition temper-



ature can be higher than, lower than or equal to the  $T_m$ . If surface lipids melted below  $T_c$ , it would demonstrate that melting can occur without affecting permeability and would thereby disprove this model. No such examples occur in Table 1. Instead, in most species the cuticular lipids melt at approximately the same temperature as the  $T_c$ . This correlation is consistent with the lipid melting model, although it must be emphasized that correlations between these parameters rely on the assumptions that lipid properties and cuticular permeability are uniform across the cuticle, neither of which is likely to be true. If cuticular properties are not uniform, one may find that transpiration increases rapidly in one part of the animal, whereas measurements of  $T_m$  in bulk lipid extracts will indicate that  $T_m$  is greater than  $T_c$ . This scenario may explain the high  $T_m$  values, relative to  $T_c$ , in *Pieris* and *T. molitor* larvae (Table 1), or thermal damage to non-lipid components of the cuticle could increase permeability. Thus, the data in Table 1 are consistent with the lipid-melting model, but can not be taken as conclusive evidence. It is possible that the lipid-melting model is incorrect, and that the apparent relationship between lipid phase behavior and cuticular transpiration will disappear as additional species are studied.

Table 1 also shows that cuticular lipids can melt at temperatures above a species' lethal limits, so that phase transitions would be ecologically irrelevant. Is there an adaptive explanation for this, or do some species simply produce lipids which happen to remain solid under ambient conditions? The two species with the highest  $T_m$  values are also known to have relatively low rates of water loss at lower temperatures (Table 3.1 in Hadley, 1994a). This suggests that high  $T_m$  values may be associated with reduced cuticular permeability at lower temperatures. The assumption that this is generally true is often made in studies of cuticular lipids: longer chain lengths mean higher melting temperatures, which mean lower rates of water loss. However, species which lose water slowly also tend to have greater quantities of surface lipid, which will reduce permeability even if lipid properties do not differ. In the absence of data on lipid

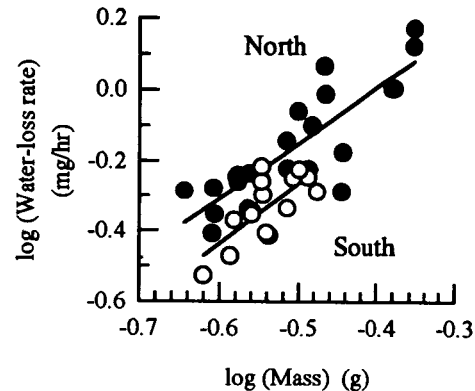


FIG. 6. Water loss rates of live grasshoppers (*Melanoplus sanguinipes*) from two California USA populations. Cuticular lipids from the southern population melt at higher temperatures than those from the northern population (B. C. Rourke, personal communication).

quantity, no conclusions can be made regarding the relationship between water loss and high- $T_m$  lipids.

In a few species, lipid amounts,  $T_m$ , and rates of water loss have all been determined. California populations of *M. sanguinipes* exhibit significant geographic variation in cuticular lipid properties (Gibbs *et al.*, 1991). The midpoint of the solid-fluid phase transition occurs at 38–50°C, which overlaps the range of body temperatures found in the field (Chappell, 1983; Rourke, personal communication). Recent work indicates that lower  $T_m$  values in northern populations are associated with higher rates of water loss, even at 25°C (Fig. 6). These populations do not differ in surface lipid quantity. Thus, even below the melting temperature, higher  $T_m$  values are correlated with reduced cuticular permeability.

Additional support for a correspondence between  $T_m$  and water loss comes from laboratory-selected populations of *D. melanogaster*. Selection for desiccation resistance has resulted in flies which lose water 40% less rapidly than unselected control populations and can survive in dry air for over two days (Gibbs *et al.*, 1997). Several physiological mechanisms are involved in the evolution of increased desiccation resistance, including changes in water content, respiratory patterns (Williams and Bradley, 1997) and cuticular lipids. Lipid amounts

do not differ between selected and control populations, but  $T_m$  values of selected populations are 1–3°C higher than those of unselected control populations (Gibbs *et al.*, 1997). These results also suggest that  $T_m$  is negatively correlated with transpiration, although it is difficult to imagine how such a dramatic difference in water loss could be attributed entirely to such a minor difference in lipid properties.

These examples suggest that solid-phase lipid layers with similar thicknesses can differ in their permeabilities. The physical basis for this observation is unclear. The molecular motion of lipid molecules will decrease with temperature even in the solid state, which may make diffusion of water more difficult. Studies using NMR are consistent with this idea (Toolson *et al.*, 1979), but additional biophysical studies are needed.

#### SUMMARY

It has been clear for decades that cuticular lipids provide the primary barrier to evaporative water loss from terrestrial arthropods, and transitions in water loss ("critical" temperatures) have been ascribed to melting of the surface lipids. Despite limited correlative evidence, this model has gained textbook status (*e.g.*, Randall *et al.*, 1997, p. 576). Recent advances in lipid biophysical methods and humidity measurement systems have now made it possible to investigate this model more directly.

Melting temperatures of cuticular lipids depend on their composition. For the major class, hydrocarbons, the most important structural feature affecting  $T_m$  is unsaturation. Introduction of a double bond can decrease the  $T_m$  of pure compounds by 50°C, and interactions between saturated and unsaturated hydrocarbons, and/or phase separation between them, result in non-linear effects of unsaturation on  $T_m$  (Fig. 3). Methylbranching also reduces  $T_m$  significantly (Fig. 2), whereas changes in chain length have much less effect.

Recent evidence (Fig. 4, 5) supports the lipid melting model to explain the critical temperature phenomenon. In model cuticle experiments, *in vitro* preparations and many

intact insects, the transition temperature for water loss is approximately equal to the  $T_m$ . Thus, variation in lipid composition, in so far as it affects lipid melting, will change the value of  $T_c$ . Studies with grasshoppers and dipterans suggest that differences in lipid  $T_m$  values may also be correlated with water loss rates at temperatures below  $T_m$ . Differences in lipid amount are also likely to be important in determining water loss rates below  $T_c$ , where transpiration is low and relatively unaffected by temperature.

An intriguing question is why surface lipids should melt at physiologically relevant temperatures. Insects from diverse taxa produce surface lipids (especially *n*-alkanes) which melt above 50°C; why don't all terrestrial arthropods minimize the risk of dehydration by synthesizing only these compounds? One possibility is the need for communication. The other major role of surface lipids is as pheromones and kairomones; thus, many attempts to explain cuticular lipid diversity have focused on chemical ecology (Howard, 1993; Singer, 1998). Many compounds used for communication are unsaturated or branched, which will tend to decrease  $T_m$ . Melting of surface lipids at high environmental temperatures may be a consequence of the need to interact with colony members or potential mates. Although pheromone components can comprise a major fraction of the total surface lipid (Nelson *et al.*, 1981; Scott, 1994), researchers interested in either behavior or physiology have generally ignored each other's fields. A few steps towards integrating these studies have begun (Markow and Toolson, 1990; Gibbs *et al.*, 1995), but much remains to be done.

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#### REFERENCES

- Bartelt, R. J., M. T. Arnold, A. M. Schaner, and L. L. Jackson. 1986. Comparative analysis of cuticular

- hydrocarbons in the *Drosophila virilis* species group. *Comp. Biochem. Physiol. B* 83:731–742.
- Beament, J. W. L. 1945. The cuticular lipoids of insects. *J. Exp. Biol.* 21:115–131.
- Beament, J. W. L. 1958. The effect of temperature on the water-proofing mechanism of an insect. *J. Exp. Biol.* 35:494–519.
- Beament, J. W. L. 1964. The active transport and passive movement of water in insects. *Adv. Insect Physiol.* 2:67–129.
- Berry, R. S., S. A. Rice and J. Ross. 1980. *Physical chemistry*. Wiley and Sons, New York.
- Blomquist, G. J., D. R. Nelson, and M. de Renobales. 1987. Chemistry, biochemistry, and physiology of insect cuticular lipids. *Arch. Insect Biochem. Physiol.* 6:227–265.
- Bonsor, D. H. and D. Bloor. 1977. Phase transitions of *n*-alkane systems. *J. Mater. Sci.* 12:1559–1564.
- Buckner, J. S. 1993. Cuticular polar lipids of insects. In D. W. Stanley-Samuelson and D. R. Nelson (eds.), *Insect lipids: Chemistry, biochemistry and biology*, pp. 227–270. University of Nebraska Press, Lincoln Nebraska.
- Cevc, G. 1991. Isothermal lipid phase transitions. *Chem. Phys. Lipids* 57:293–307.
- Chappell, M. A. 1983. Metabolism and thermoregulation in desert and montane grasshoppers. *Oecologia* 56:126–131.
- Chefurka, W. and J. H. Pepper. 1955. Studies on the cuticle of the grasshopper *Melanoplus bivittatus* (Say) (Orthoptera: Acrididae). III. On the physical nature of the transition region in insect waxes. *Canad. Entomologist* 87:163–171.
- Davis, M. B. 1974. Critical temperature and changes in cuticular lipids in the rabbit tick, *Haemaphysalis leporispalustris*. *J. Insect Physiol.* 20:1087–1100.
- Crowe, J. H., F. A. Hoekstra and L. M. Crowe. 1992. Anhydrobiosis. *Annu. Rev. Physiol.* 54:579–599.
- Edney, E. B. 1977. *Water balance in land arthropods*. Springer-Verlag, Berlin.
- Gelman, N. and J. Machin. 1994. Diffusion through the water barrier of arthropod cuticles: A statistical mechanical approach to the analysis of temperature effects. *J. Theor. Biol.* 167:361–372.
- Gelman, N., J. Machin and P. Kestler. 1988. The nature of driving forces for passive water transport through arthropod cuticle. *J. Therm. Biol.* 13:157–162.
- Gibbs, A. 1995. Physical properties of insect cuticular hydrocarbons: Model mixtures and interactions. *Comp. Biochem. Physiol. B* 112:667–672.
- Gibbs, A. and J. H. Crowe. 1991. Intra-individual variation cuticular lipids studied using Fourier transform infrared spectroscopy. *J. Insect Physiol.* 37:743–748.
- Gibbs, A. G., A. K. Chippindale, and M. R. Rose. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* 200:1821–1832.
- Gibbs, A., M. Kuenzli, and G. J. Blomquist. 1995. Sex- and age-related changes in the biophysical properties of cuticular lipids of the housefly, *Musca domestica*. *Arch. Insect Biochem. Physiol.* 29:87–97.
- Gibbs, A. G., A. K. Louie, and J. A. Ayala. 1998. Effects of temperature on cuticular lipids and water balance in desert *Drosophila*: Is thermal acclimation beneficial? *J. Exp. Biol.* 201:71–80.
- Gibbs, A. and T. A. Mousseau. 1994. Thermal acclimation and genetic variation in cuticular lipids of the lesser migratory grasshopper (*Melanoplus sanguinipes*): Effects of lipid composition on biophysical properties. *Physiol. Zool.* 67:1523–1543.
- Gibbs, A., T. A. Mousseau, and J. H. Crowe. 1991. Geographic and acclimatory variation in insect cuticle lipids. *Proc. Natl. Acad. Sci. U.S.A.* 88:7257–7260.
- Gibbs, A. and J. G. Pomonis. 1995. Physical properties of insect cuticular hydrocarbons: The effects of chain length, methyl-branching and unsaturation. *Comp. Biochem. Physiol. B* 112:243–249.
- Hadley, N. F. 1977. Epicuticular lipids of the desert tenebrionid beetle, *Eleodes armata*: Seasonal and acclimatory effects on composition. *Insect Biochem.* 7:277–283.
- Hadley, N. F. 1978. Cuticular permeability of desert tenebrionid beetles: Correlations with epicuticular hydrocarbon composition. *Insect Biochem.* 8:17–22.
- Hadley, N. F. 1994a. *Water relations of terrestrial arthropods*. Academic Press, San Diego, California.
- Hadley, N. F. 1994b. Ventilatory patterns and respiratory transpiration in adult terrestrial insects. *Physiol. Zool.* 67:175–189.
- Hadley, N. F. and M. C. Quinlan. 1987. Permeability of arthroal membrane to water: First measurement using *in vivo* techniques. *Experientia* 43:164–166.
- Hadley, N. F. and M. C. Quinlan. 1989. Cuticular permeability of the black widow spider *Latrodectus hesperus*. *J. Comp. Physiol. B* 159:243–248.
- Hadley, N. F. and T. D. Schultz. 1987. Water loss in three species of tiger beetles (*Cicindela*): Correlations with epicuticular hydrocarbons. *J. Insect Physiol.* 33:677–682.
- Hadley, N. F., J. L. Stuart, and M. C. Quinlan. 1982. An air-flow system for measuring total transpiration and cuticular permeability in arthropods: Studies on the centipede *Scolopendra polymorpha*. *Physiol. Zool.* 55:393–404.
- Hadley, N. F., E. C. Toolson, and M. C. Quinlan. 1989. Regional differences in cuticular permeability in the desert cicada *Diceroprocta apache*: Implications for evaporative cooling. *J. Exp. Biol.* 141:219–230.
- Holdgate, M. W. and M. Seal. 1956. The epicuticular wax layers of the pupa of *Tenebrio molitor* L. *J. Exp. Biol.* 33:82–106.
- Howard, R. W. 1993. Cuticular hydrocarbons and chemical communication. In D. W. Stanley-Samuelson and D. R. Nelson (eds.), *Insect lipids: Chemistry, biochemistry and biology*, pp. 179–226. University of Nebraska Press, Lincoln Nebraska.
- Howard, R. W., C. D. Howard, and S. Colquhoun. 1995. Ontogenetic and environmentally induced

- changes in cuticular hydrocarbons of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae). *Ann. Entomol. Soc. Am.* 88:485-495.
- Lighton, J. R. B. 1994. Discontinuous ventilation in terrestrial insects. *Physiol. Zool.* 67:142-162.
- Lighton, J. R. B. and D. H. Feener. 1989. Water-loss rate and cuticular permeability of the desert ant *Pogonomyrmex rugosus*. *Physiol. Zool.* 62:1232-1256.
- Lockey, K. H. 1976. Cuticular hydrocarbons of *Locusta*, *Schistocerca*, and *Periplaneta*, and their role in waterproofing. *Insect Biochem.* 6:457-472.
- Lockey, K. H. 1988. Lipids of the insect cuticle: Origin, composition and function. *Comp. Biochem. Physiol. B* 89:595-645.
- Lockey, K. H. 1991. Insect hydrocarbon classes: Implications for chemotaxonomy. *Insect Biochem.* 21:91-97.
- Loveridge, J. P. 1968. The control of water loss in *Locusta migratoria migratorioides* R. & F. I. Cuticular water loss. *J. Exp. Biol.* 49:1-13.
- Loveridge, J. P. 1980. Cuticular water relations techniques. *In* T. A. Miller (ed.), *Cuticle techniques in arthropods*, pp. 301-366. Springer-Verlag, Berlin.
- Machin, J. and G. J. Lampert. 1989. Energetics of water diffusion through the cuticular water barrier of *Periplaneta*: The effect of temperature, revisited. *J. Insect Physiol.* 35:437-445.
- Machin, J. and G. J. Lampert. 1990. Evidence for water-organized structure in the cuticular water barrier of the American cockroach, *Periplaneta americana*. *J. Comp. Physiol. B* 159:739-744.
- Markow, T. A. and E. C. Toolson. 1990. Temperature effects on epicuticular hydrocarbons and sexual isolation in *Drosophila mojavensis*. *In* J. S. F. Barker, W. T. Starmer, and R. J. MacIntyre (eds.), *Ecological and evolutionary genetics of Drosophila*. Plenum, New York.
- Maroncelli, M., S. P. Qi, H. L. Strauss, and R. G. Snyder. 1982. Nonplanar conformers and the phase behavior of solid *n*-alkanes. *J. Am. Chem. Soc.* 104:6237-6247.
- Monteith, J. L. and G. S. Campbell. 1980. Diffusion of water vapor through integuments- potential confusion. *J. Therm. Biol.* 5:7-9.
- Nelson, D. R., J. W. Dillwith, and G. J. Blomquist. 1981. Cuticular hydrocarbons of the housefly, *Musca domestica*. *Insect Biochem.* 11:187-197.
- Ramsay, J. A. 1935. The evaporation of water from the cockroach. *J. Exp. Biol.* 12:373-383.
- Randall, D. J., W. Burggren, and K. French. 1997. *Eckert animal physiology: Mechanisms and adaptations*. W. H. Freeman and Co., New York.
- de Renobales, M., D. R. Nelson, and G. J. Blomquist. 1991. Cuticular lipids. *In* K. Binnington and A. Retnakaran (eds.), *Physiology of the insect epidermis*, pp. 242-253. CSIRO Publications, East Melbourne Australia.
- Scott, D. R. 1994. Genetic variation for mate discrimination in *Drosophila melanogaster*. *Evolution* 48: 112-121.
- Singer, T. L. 1998. The roles of hydrocarbons in the recognition systems of insects. *Amer. Zool.* 38: 394-405.
- Small, D. M. 1986. *The physical chemistry of lipids: From alkanes to phospholipids. Handbook of lipid research*, Vol. 4. Plenum Press, New York.
- Stanley-Samuelson, D. W. and D. R. Nelson. (eds.) 1993. *Insect lipids: Chemistry, biochemistry and biology*. University of Nebraska Press, Lincoln, Nebraska.
- Toolson, E. C. 1978. Diffusion of water through the arthropod cuticle: Thermodynamic consideration of the transition phenomenon. *J. Therm. Biol.* 3: 69-73.
- Toolson, E. C. 1980. Thermodynamic and kinetic aspects of water flux through the arthropod cuticle. *J. Therm. Biol.* 5:1-6.
- Toolson, E. C. 1982. Effects of rearing temperature on cuticle permeability and epicuticular lipid composition in *Drosophila pseudoobscura*. *J. Exp. Zool.* 222:249-253.
- Toolson, E. C. 1984. Interindividual variation in epicuticular hydrocarbon composition and water loss rates of the cicada *Tibicen dealbatus* (Homoptera: Cicadidae). *Physiol. Zool.* 57:550-556.
- Toolson, E. C. and N. F. Hadley. 1977. Cuticular permeability and epicuticular lipid composition in two Arizona vejovid scorpions. *Physiol. Zool.* 50: 323-330.
- Toolson, E. C. and N. F. Hadley. 1979. Seasonal effects on cuticular permeability and epicuticular lipid composition in *Centruroides sculpturatus* Ewing 1928 (Scorpiones: Buthidae): Correlation with thermal effects on cuticular permeability. *J. Insect Physiol.* 25:271-275.
- Toolson, E. C., T. R. White, and W. S. Glaunsinger. 1979. Electron paramagnetic resonance spectroscopy of spin-labelled cuticle of *Centruroides sculpturatus* (Scorpiones: Buthidae): Correlation with thermal effects on cuticular permeability. *J. Insect Physiol.* 25:271-275.
- Wigglesworth, V. B. 1945. Transpiration through the cuticle of insects. *J. Exp. Biol.* 21:97-114.
- Williams, A. E. and T. J. Bradley. 1997. CO<sub>2</sub> release patterns in *Drosophila melanogaster*: The effects of selection for desiccation resistance. *J. Exp. Biol.* 200:615-624.